R.A.P.I.D. Workshop

RUGGEDIZED > ADVANCED > PATHOGEN > IDENTIFICATION > DEVICE

Idaho Technology Inc.

>>> Basic Training Workbook

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R.A.P.I.D.™ Workshop/User's Workbook - March 2001

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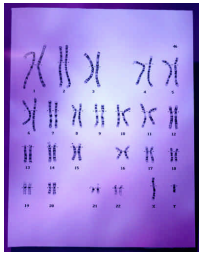
The R.A.P.I.D. can be used for PCR as well as other methods. PCR is a proprietary technology covered by several US patents including US Patent Nos. 4,683,195, 4,683,202 and 4,965,188, and by issued and pending counterparts outside the U.S. These patents are owned by Roche Molecular Systems Inc (RMS) and have been sub-licensed by PE Corporation (PE) in certain fields. Depending on your specific application you may need a license from RMS or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA94501 or Applied Biosystems (a.k.a. PE), 850 Lincoln Centre Drive, Foster City, CA94404.

Version: 11/16/01

Polymerase Chain Reaction (PCR) is a technique used to amplify DNA. PCR is the most specific and sensitive assay available. From a single DNA molecule and specific building blocks, subsequent DNA copies can be made. The R.A.P.I.D. offers the most advanced and easy-to-use real-time PCR detection system available today.

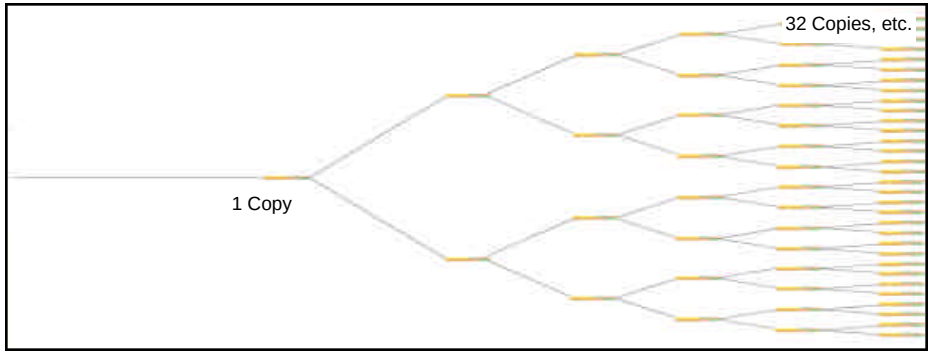
Applications

Identifying biowarfare agents, clinical diagnosis, genetic studies (mutations, paternity), criminal identifications



Theory

After each round or cycle, DNA copies are made at an exponential rate. Starting with1 copy of DNA, 2 copies are made after one cycle, 4 after two cycles, etc. After 30 cycles, PCR will produce over a billion DNA copies that can then be measured.



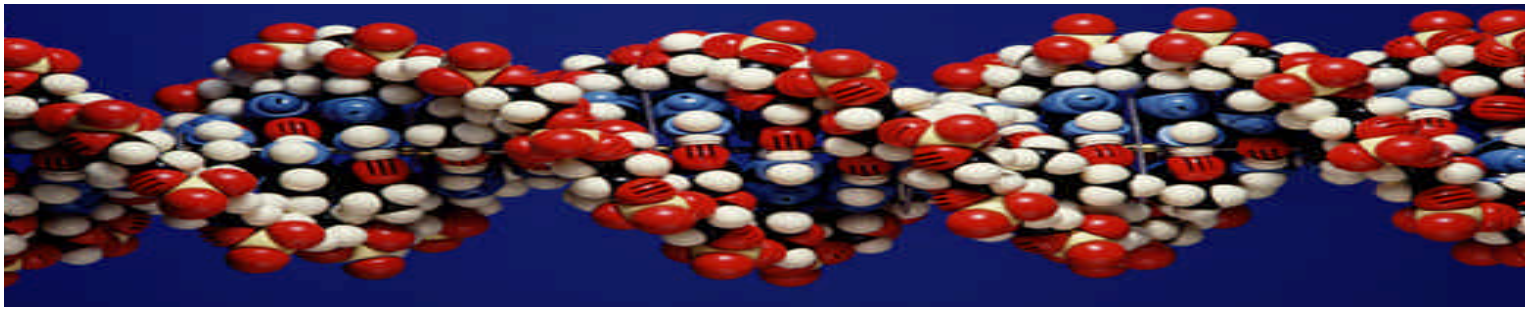
Did You Know...

The polymerase chain reaction was invented in 1983 by Kary Mullis of Cetus Corporation, and since then it has become an extremely important molecular biology technique with direct applications in genetic research, medi - cine and forensics. PCR is such a leap forward in molecular biology techniques that Kary Mullis shared the Nobel prize in chemistry in 1993.

NOTES:

Ingredients

Elements for PCR include **sample DNA**, **primers**, **probes**, **dNTP** molecules, **Taq** polymerase enzyme, and **buffer**.



Sample DNA provides the target template.

Primers are short fragments specific to the DNA target. The primers start the DNA synthesis.

Probes are also DNA specific and have fluorescent dyes attached to their sequences.

dNTPs (nucleotide bases) are the actual building blocks used to create the copied DNA strand. There are four nucleotide bases: adenine (A), cytosine (C), guanine (G), and thymine (T). Adenine always pairs with thymine and cytosine always pairs with guanine.

Taq polymerase enzyme builds the DNA copy by placing each nucleotide base in its proper position.

Buffer maintains the reaction pH (a measure of the strength of acids and bases).

****IT BioReagents contain all the ingredients for PCR in a ready-to-use product.**

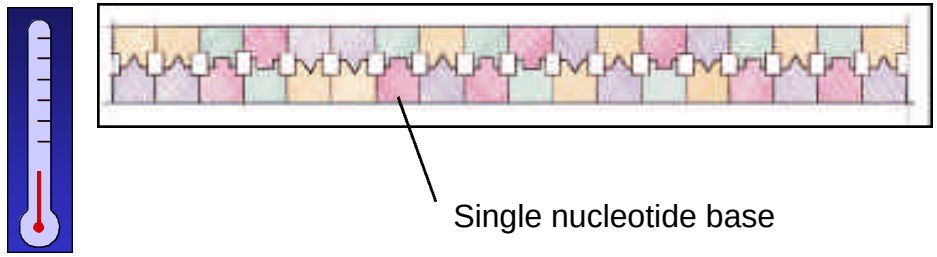
NOTES:

Temperature Cycling

DNA is a molecule found in all living cells and certain viruses (animal, plant, bacteria, fungal, etc.). Each DNA is specific among species. In nature (for example, ambient temperature), DNA exists as a double stranded molecule (helix). PCR requires temperature cycling (heating and cooling) to make subsequent DNA copies.

Double stranded DNA in nature

Room Temperature 25º C



Did You Know...

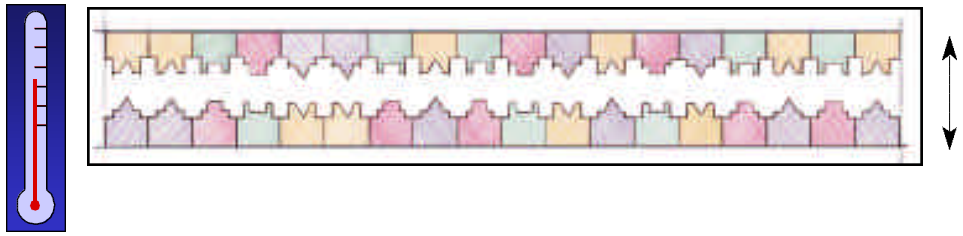
To have an idea of the size of DNA, every million bases take up a linear space of 0.34 mm. So when you take one human cell and uncoil all the chromo - somal DNA and put it on a line, you would have 204 cm of DNA. (A human cell contains a total of 6 x 109 nucleotide pairs). To store this information on paper, you need a few pages of 8.5x11 paper: one page stores 3100 letters on one side, so 6 x 109 nucleotides, printed on both sides of a sheet, would need 967,742 pages.

One cycle or round of PCR usually consists of 3 steps:

1. Denaturation: Usually 94º C to separate the double stranded DNA molecule

DNA separating into single strands

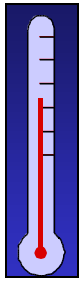
94º C



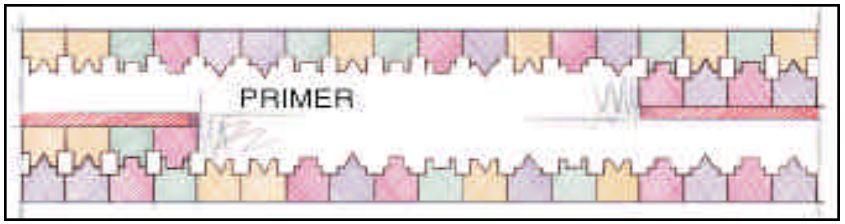
NOTES:

2. Annealing: A lower temperature between 50-68º C where the primers bind to the separated DNA strands

60º C

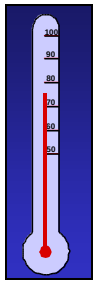


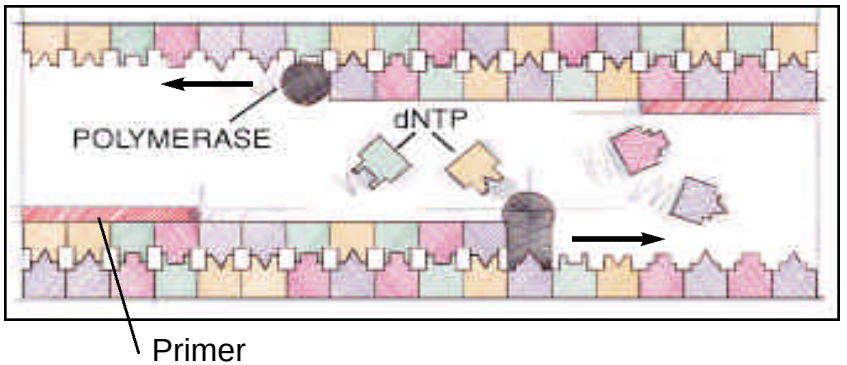
Primers bind to single DNA strands



3. Extension: The temperature that allows the Taq enzyme to create the DNA copy.

72º C





A typical PCR program will run a total of 30-50 cycles.
In air-thermocycling, it is possible to combine the annealing and extensions steps into one single step.

Did You Know...

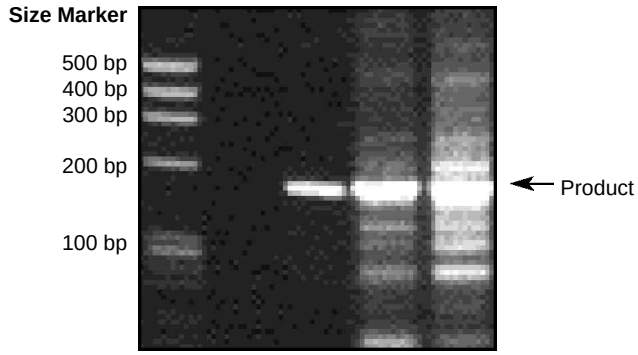
Because both strands of DNA are copied during PCR, there is an exponential increase of the number of copies of the gene.

Suppose there is only one copy of the wanted gene before the cycling starts, after one cycle, there will be 2 copies, after two cycles, there will be 4 copies, three cycles will result in 8 copies and so on. After 35 cycles, you will have nearly 34 billion copies.

NOTES:

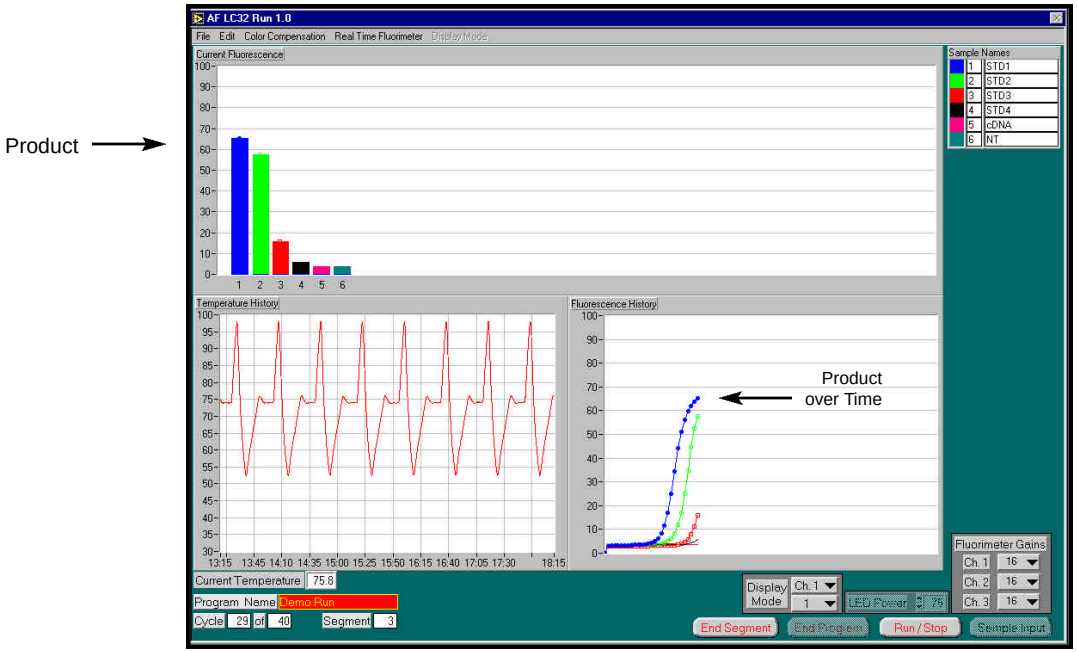
DNA Product

Once cycling is complete, the user needs a tool to view the product. Typically this is an agarose gel viewed under a UV lamp.



Real-Time PCR

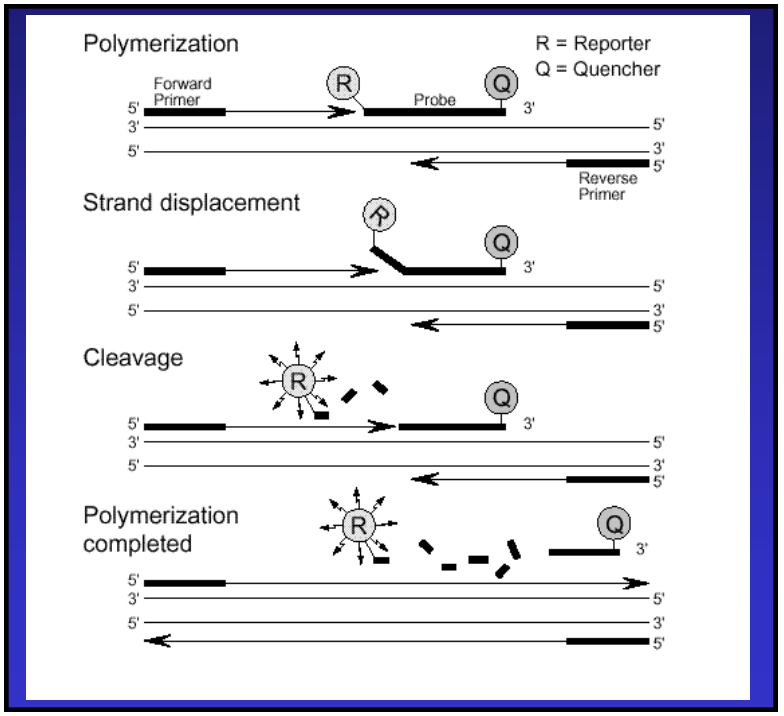
In real-time PCR, a fluorescent dye or probe is used to measure product. These probes are also specific to the target DNA and the product is measured by the R.A.P.I.D. fluorimeter.



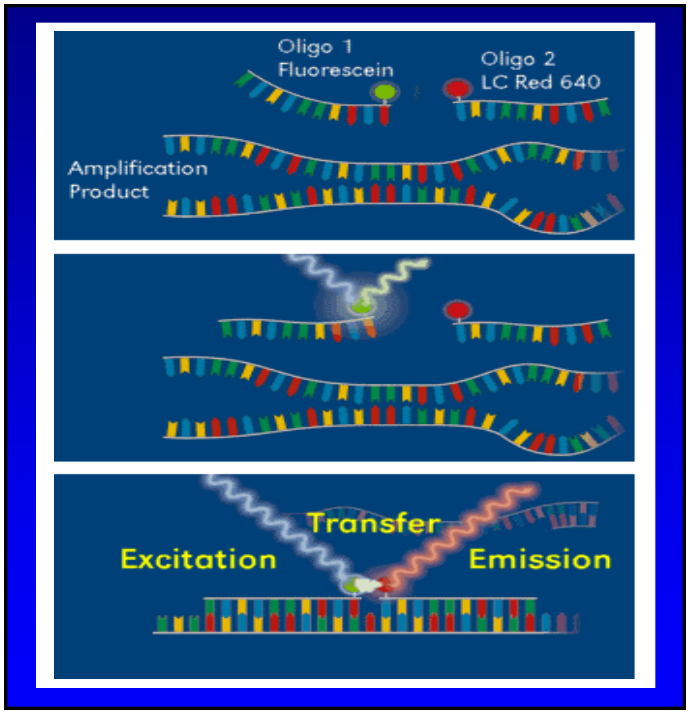
NOTES:

Probes with fluorescent dyes are used for Real-Time PCR

Hydrolysis Probe



Hybridization Probe

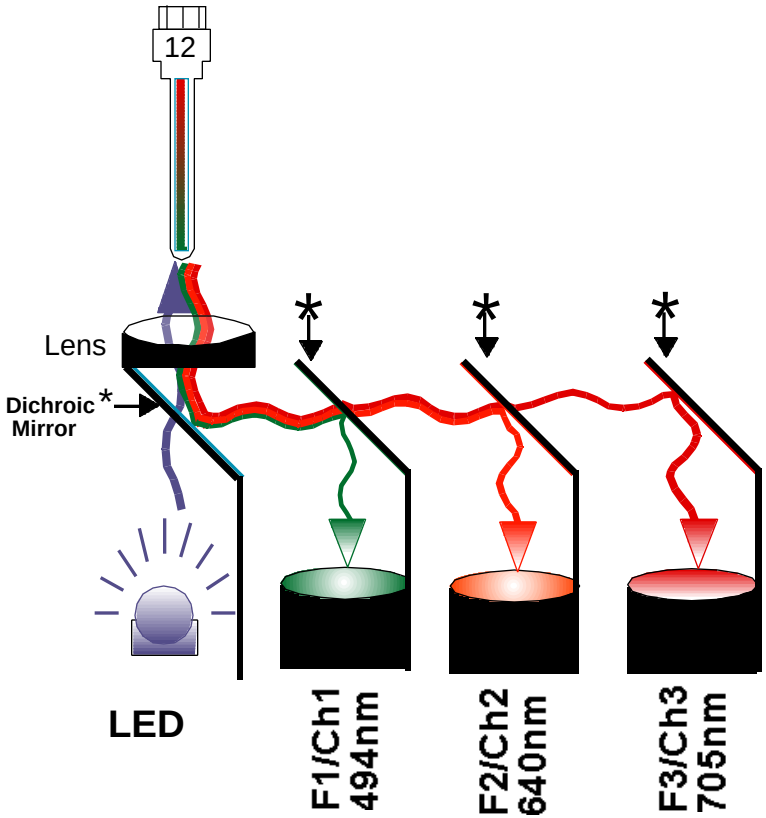


NOTES:

R.A.P.I.D. System Fluorescent Detecting Optics

For fluorescence excitation the R.A.P.I.D. instrument is equipped with a blue LED light source which emits light. This LED, which is an integral part of the fluorimeter, needs no maintenance and has a long life span.

The fluorimeter provides three channels which detect emitted light with varing wavelengths. Due to the high quality optics the measurement is both extremely fast and sensitive. In a normal amplification run, measurement of a sample only takes about 20 milliseconds.



NOTES:

R.A.P.I.D. System

NOTES:

Unpacking the R.A.P.I.D.:

1. Unzip and remove R.A.P.I.D. from case.

2. Release pressure button and unbuckle latches.

3. Connect power cords for laptop computer and R.A.P.I.D.

4. Connect computer cord between R.A.P.I.D. and laptop computer.

5. Remove white thermoguard ring (this protects thermocouple).

NOTES:

User Access/Security:

Logging-on to Laptop Computer

Access to Advanced Options

User Name: Administrator
Password: {empty password}

No Advanced Options

User Name: Guest
Password: {empty password}

Assigning New User Access
(R.A.P.I.D. Technical Manual, Chapter 5 (User Access), p. 31.

Follow the directions to add your name as part of the RapidUsers Group

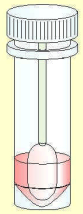
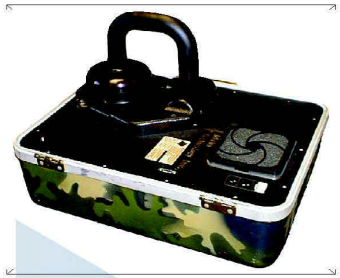
Username _____

Password _____

NOTES: _____

Laboratory Work Flow to Minimize Contamination

Lab Area

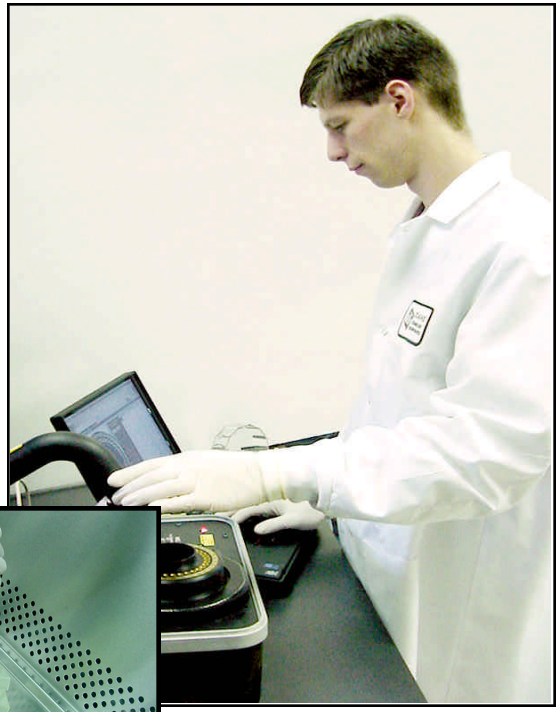
Outside Collection	Room/Area 1	Room/Area 2	Room/Area 3
Sample Collection	**Sample Prep**	**Reaction Prep**	**Run Reaction**
			
Supplies Needed	**Supplies Needed**	**Supplies Needed**	**Supplies Needed**
Bead Tube Swab from IT 1-2-3 Kit	IT 1-2-3 Kit P-1000 Pipettor Bead Beater Centrifuge	IT Freeze-Dried Reagents P-200 and P-20 Pipettors Capillary Cuvettes Centrifuge	R.A.P.I.D. Instrument Laptop with R.A.P.I.D. Software

Be sure to follow labortary flow to minimize contamination.

NOTES:

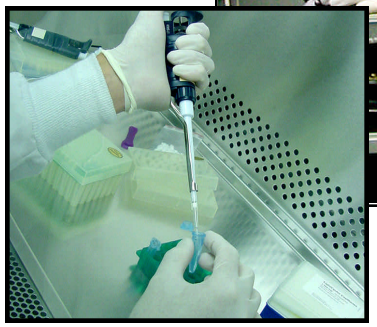
General Lab Practices:

No eating, drinking or smoking in lab area!!!



Use a designated lab area to minimize contamination to sample.

When handling samples, wear and change gloves frequently.



NOTES:

Pipetting (Gilson pipettors)

a: plunger button volume adjustment

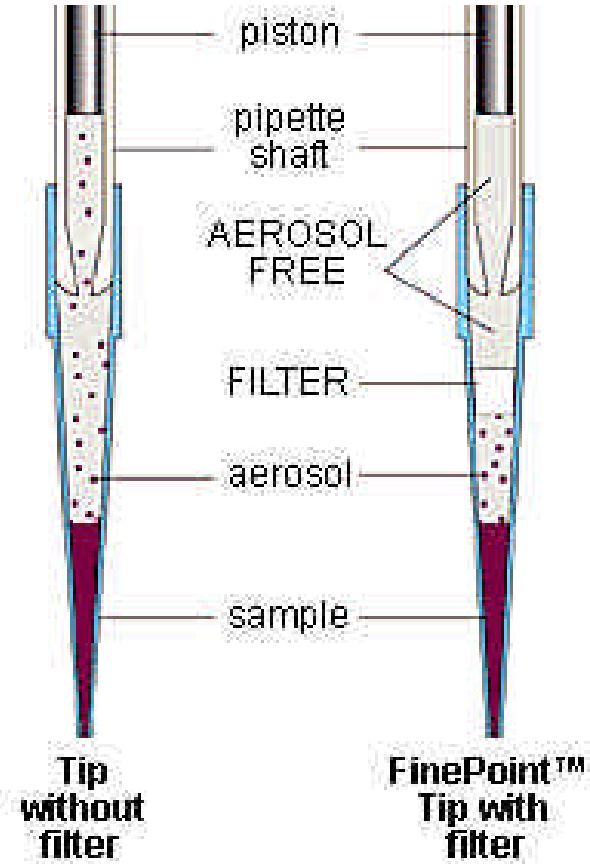
b: knurled knob volume adjustment

c: non-slip tip ejector, easy on - easy off

NOTES:

Pipetting Tips

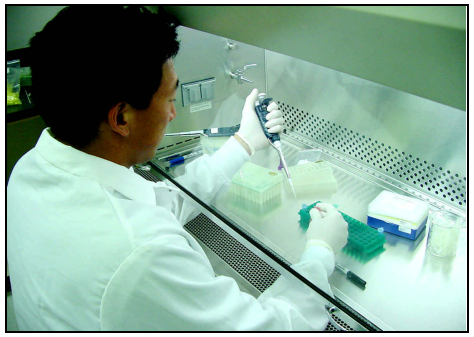
- Since small volumes are measured, draw liquid slowly to avoid air bubbles. Do the same when expelling liquid.
- Practice pipetting by transferring water to parafilm. Note volume sizes.
- Always use aerosol-barrier pipette tips (PCR certified)
- 3 sizes of pipette tips used (3 Gilson pipettors)
  - P-20 (measures 2-20 µl)
  - P-200 (measures 20-200 µl)
  - P-1000 (measures 100-1000 µl)

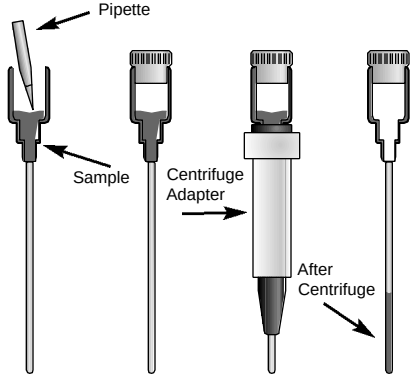


NOTES:

Reagent Setup

Technician / Operator





A 5 - 20 µl sample is pipetted into a **capillary tube**, centrifuged to place the sample at the tip of the reaction cuvette for fluorescence monitoring.

IT BioReagents

Capillary tubes in microcentrifuge

Be certain that the centrifuge adapters are placed in a balanced arrangement in the centrifuge.

NOTES:

DNA Sample Preparation

Introduction

DNA is present in microorganisms that compose clinical and environmental samples, including soil, water, blood, etc. DNA extraction and purification from a complex sample is required to run R.A.P.I.D. PCR identification. Besides the associated debris and media, there are many chemical inhibitors present in the sample. Certain chemicals will break down DNA, thus hindering the PCR reaction.

Typical sample preparation involves two steps:

First, the **DNA IS EXTRACTED** from the microorganisms in the sample (e.g. cells or spores). This is achieved by physical agitation and disruption of the cells. IT employs the bead-beating method.

Second, the **DNA IS PURIFIED** and concentrated using a commercial kit. Below is a typical sample preparation procedure:

Biospec
Bead Beater

NOTES:

Bead Beating: (Extraction)

Material required

IT 1-2-3 DNA Purification Kit which includes swabs, buffers, bead tubes, spin columns and centrifuge tubes. Additionally you will need pipettors (P20, P200 and P1000), disposable pipette tips, a R.A.P.I.D. System, Micro-Centrifuge and Bio-Spec Mini Bead Beater. Be sure to wear gloves and protective gear throughout this procedure. Discard all used disposables into a biosafety waste container. We recommend that you divide the buffers into small containers to control possible contamination.

Bead Beating: (Extraction)

1. Dip swab in a bead tube and make sure the swab is wet with lysis buffer.
2. Swab the affected area with the damp swab (i.e. swab surface).
3. Place the swab into the bead tube and snap off the swab stick end, leaving the swab in the tube.
   (alternatively; if you are using cultured samples, add 20 µl to bead tube).
4. Screw on cap tight.
5. Place bead tube in the bead beater for the specified amount of time.

Bead Beating Settings

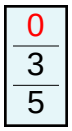
Vortex Genie 2:	Maximum Speed 5 Minutes.
BioSpec:	Speed Setting 42 (4200 rpm) Time Settings 12 (120 sec.)

Bio-Spec Mini Bead Beater

NOTES: _____

Part 1: Binding DNA to Filter

1. Set the **P-1000 pipette to 350µl**. Pipette 350µl of **Buffer 1** into the bead tube containing the swab stick end. Centrifuge briefly.



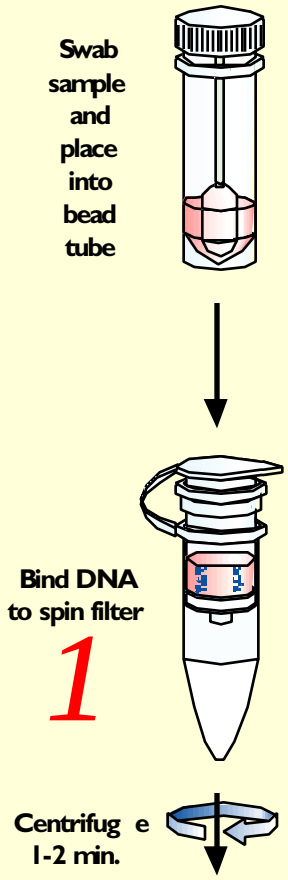
Pipette Window

2. Using the **P-1000 pipette set at 350µl** and a clean tip, draw out as much of the liquid sample from the bead tube without drawing out any of the beads and place the liquid sample into the top of the filter-receiver assembly (it is not necessary to remove all the liquid sample from the tube). Repeat if necessary.

3. Close the cap on the filter-receiver assembly. Centrifuge 1-2 minutes or until all liquid has been pulled into the bottom receiver tube.

4. Open the lid and remove the filter. Close the lid of the receiver tube. Discard the receiver tube with its contents. (DNA is captured in the filter).

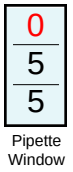
5. Place the filter into a new receiver tube.



NOTES:

Part 2: Wash Filter – DNA Clean-Up

1. Set the P-1000 to 550µl. Pipette 550µl of **Buffer 2** into the filter-receiver assembly.



Pipette Window

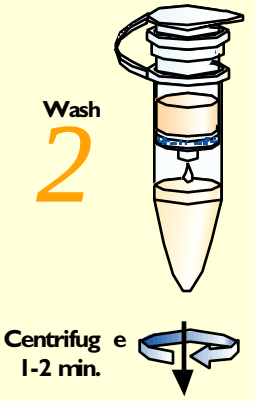
2. Close assembly lid and spin in centrifuge for 1-2 minutes or until all of the liquid is in the bottom of the receiver tube.

3. Open the lid and remove the filter. Close the lid of the receiver tube. Discard the receiver tube with its contents.

4. Place the filter into a new receiver tube and centrifuge for 3 minutes.

Note: This step is very important because the residual ethanol in Buffer 2 can hinder your PCR reaction. It is critical that all of Buffer 2 is removed from the filter.

5. Open the lid and remove the filter. Close the lid of the receiver tube. Discard the receiver tube with its contents.

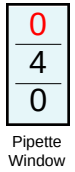


NOTES:

Part 3: DNA Elution

1. Place the filter into a new receiver tube.

2. Set the P-1000 to 400µl. Pipette 400µl of **Buffer 3*** into the filter-receiver assembly.



Pipette Window

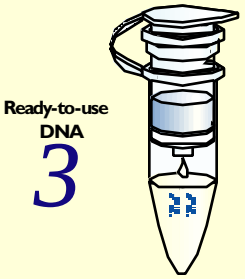
3. Close the lid of the assembly and let it sit at room temperature for 2 minutes.

4. Close assembly lid. Centrifuge for 1-2 minutes or until all of the liquid is in the bottom of the receiver tube.

5. Keep the receiver tube containing the DNA. Discard the filter.

6. Close the lid of the receiver tube. Label and date the tube containing the sample DNA. Carefully store DNA after use at 4ºC to -20ºC. This is the DNA that will be used for PCR analysis.

* For a more concentrated DNA sample, add 100 µl instead 400 µl of Buffer 3 to the filter-receiver assembly. Continue with protocol from step 3.



NOTES: _____

Using IT BioReagents

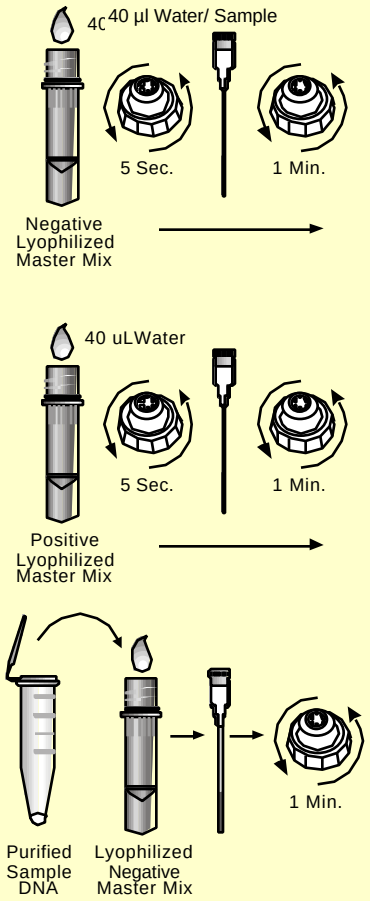
Each vial of IT BioReagents (labeled positive, negative or unknown) contains enough PCR master mix for two reactions. The vial labeled "**Positive**" contains a specified concentration of DNA to use as a positive control. The vial labeled "**Negative**" contains no target DNA to use as a negative control. Vials labeled "**Unknown**" are used to test samples.

1. Reconstitute the **Negative** and **Positive** controls with 40 µl of sterile PCR grade water. First, do the **Negative** controls and cap them to avoid cross contamination. Gently mix the contents of the master mix vial by pipetting the liquid up and down. Briefly centrifuge the master mix vial (5-10 seconds) to make sure the liquid is at the bottom. Transfer the master mix by dispensing 18 µl from the vial into the capillary tubes.

2. Reconstitute an **Unknown** IT BioReagent master mix with 40 µl of purified sample DNA. Gently mix the contents of the master mix vial by pipetting the liquid up and down. Briefly centrifuge the master mix vial (5-10 seconds) to make sure the liquid is at the bottom. Transfer the master mix by dispensing 18 µl from the vial into the capillary tubes.

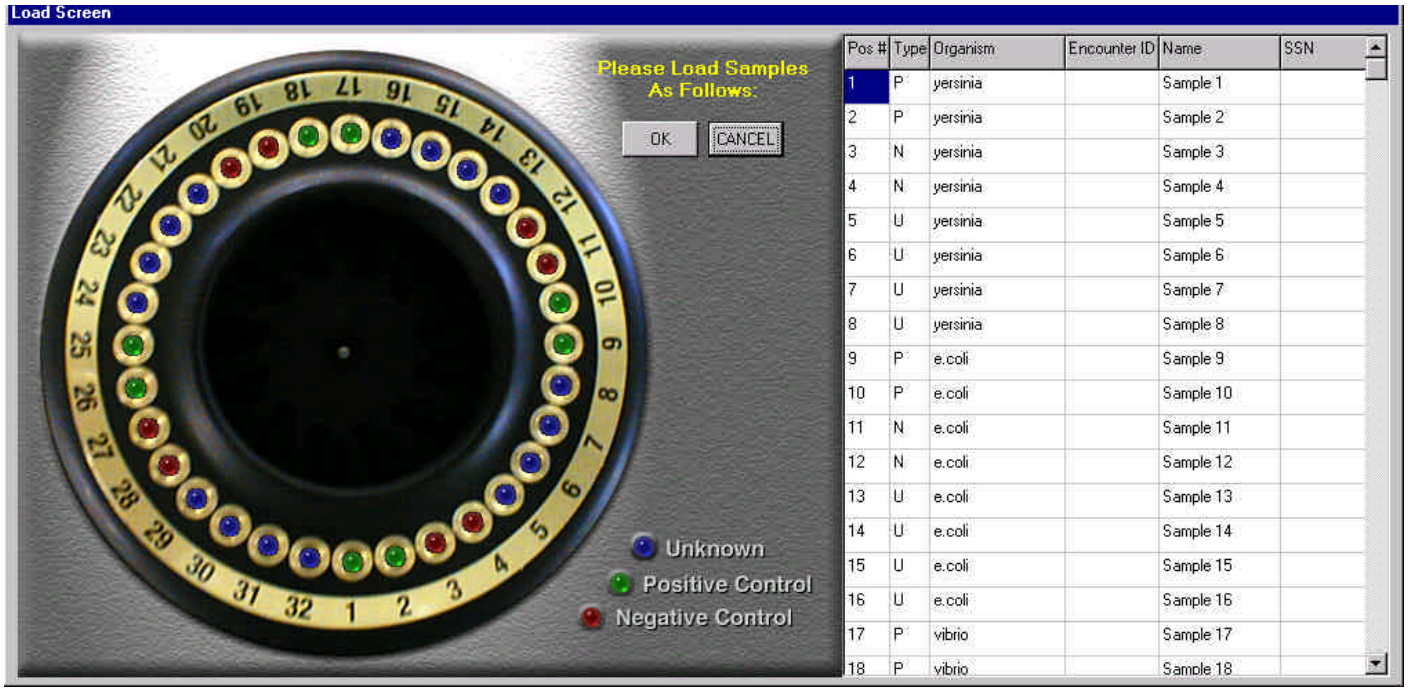
3. Repeat Step 1 using the **Positive** controls.

4. Briefly centrifuge capillary tubes.



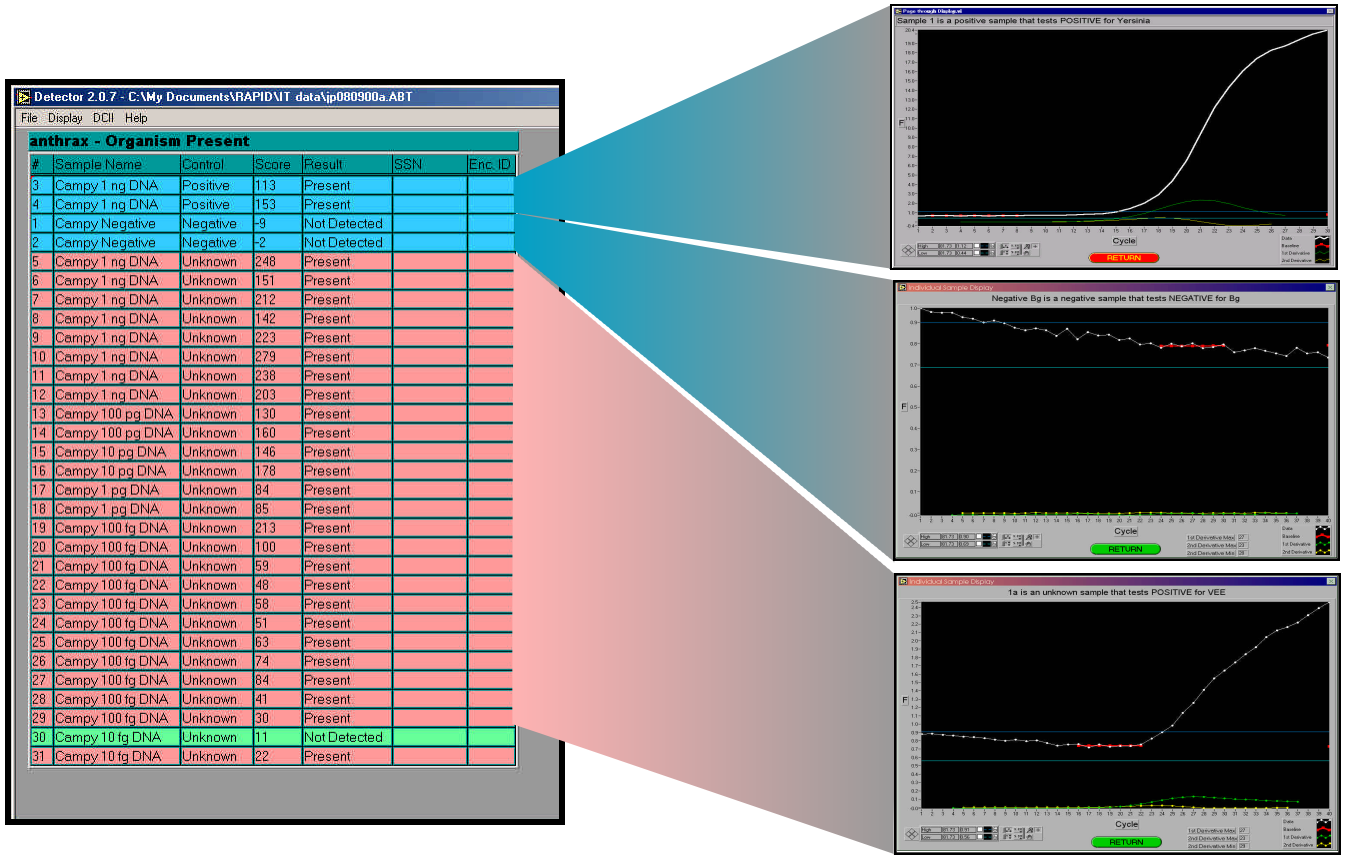
NOTES:

5. Select appropriate Test Button. Load the carousel according to the Load window (Batch or Screen test) and run the desired RAPID program.


Load Screen
Please Load Samples As Follows:
OK
CANCEL
Unknown
Positive Control
Negative Control
Pos # | Type | Organism | Encounter ID | Name | SSN
1 | P | yersinia | | Sample 1
2 | P | yersinia | | Sample 2
3 | N | yersinia | | Sample 3
4 | N | yersinia | | Sample 4
5 | U | yersinia | | Sample 5
6 | U | yersinia | | Sample 6
7 | U | yersinia | | Sample 7
8 | U | yersinia | | Sample 8
9 | P | e.coli | | Sample 9
10 | P | e.coli | | Sample 10
11 | N | e.coli | | Sample 11
12 | N | e.coli | | Sample 12
13 | U | e.coli | | Sample 13
14 | U | e.coli | | Sample 14
15 | U | e.coli | | Sample 15
16 | U | e.coli | | Sample 16
17 | P | vibrio | | Sample 17
18 | P | vibrio | | Sample 18

Detector Results Analysis

NOTES: _____


Detector 2.0.7 - C:\My Documents\RAPID\IT data\jp080900a.ABT
File Display DCII Help
anthrax - Organism Present
Sample Name Control Score Result SSN Enc. ID
3 Campy 1 ng DNA Positive 113 Present
4 Campy 1 ng DNA Positive 153 Present
1 Campy Negative Negative -9 Not Detected
2 Campy Negative Negative -2 Not Detected
5 Campy 1 ng DNA Unknown 248 Present
6 Campy 1 ng DNA Unknown 151 Present
7 Campy 1 ng DNA Unknown 212 Present
8 Campy 1 ng DNA Unknown 142 Present
9 Campy 1 ng DNA Unknown 223 Present
10 Campy 1 ng DNA Unknown 279 Present
11 Campy 1 ng DNA Unknown 238 Present
12 Campy 1 ng DNA Unknown 203 Present
13 Campy 100 pg DNA Unknown 130 Present
14 Campy 100 pg DNA Unknown 160 Present
15 Campy 10 pg DNA Unknown 146 Present
16 Campy 10 pg DNA Unknown 178 Present
17 Campy 1 pg DNA Unknown 84 Present
18 Campy 1 pg DNA Unknown 85 Present
19 Campy 100 fg DNA Unknown 213 Present
20 Campy 100 fg DNA Unknown 100 Present
21 Campy 100 fg DNA Unknown 59 Present
22 Campy 100 fg DNA Unknown 48 Present
23 Campy 100 fg DNA Unknown 58 Present
24 Campy 100 fg DNA Unknown 51 Present
25 Campy 100 fg DNA Unknown 63 Present
26 Campy 100 fg DNA Unknown 74 Present
27 Campy 100 fg DNA Unknown 84 Present
28 Campy 100 fg DNA Unknown 41 Present
29 Campy 100 fg DNA Unknown 30 Present
30 Campy 10 fg DNA Unknown 11 Not Detected
31 Campy 10 fg DNA Unknown 22 Present
Sample 1 is a positive sample that tests POSITIVE for Yersinia
Negative Bg is a negative sample that tests NEGATIVE for Bg
1a is an unknown sample that tests POSITIVE for VEE
Cycle
RETURN

Notes: Open Practice Time (Sample Prep/Reagents/PCR/Pipetting / MMBWC CD-ROM)

NOTES:

Pipette practice: Transfer the following volumes from a tube to parafilm

a. 450 µl

b. 50 µl

c. 18 µl

Run samples using IT BioReagents and pre-programmed R.A.P.I.D. program.

R.A.P.I.D. Software

NOTES: _____

Follow R.A.P.I.D. Technical Manual:

Chapter 4: Screen/Batch Tests

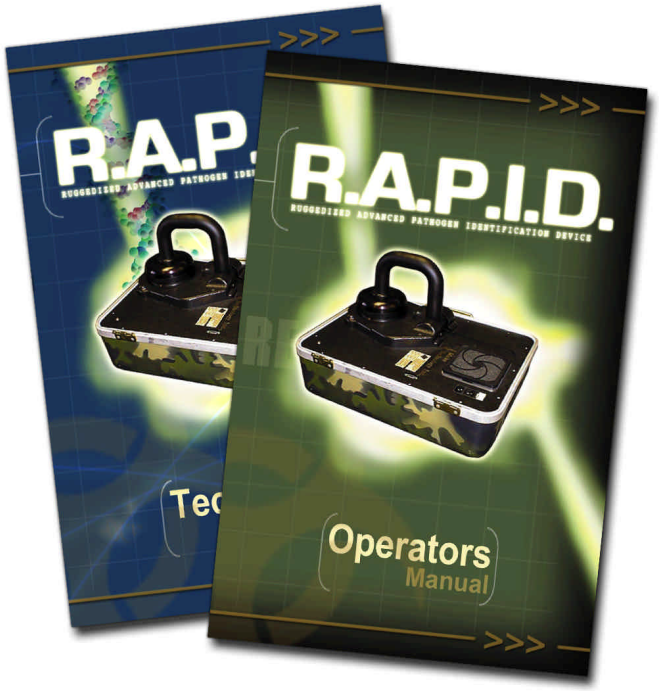
Chapter 5: Advanced Options

a. Run programming

b. Data Analysis

c. Detector

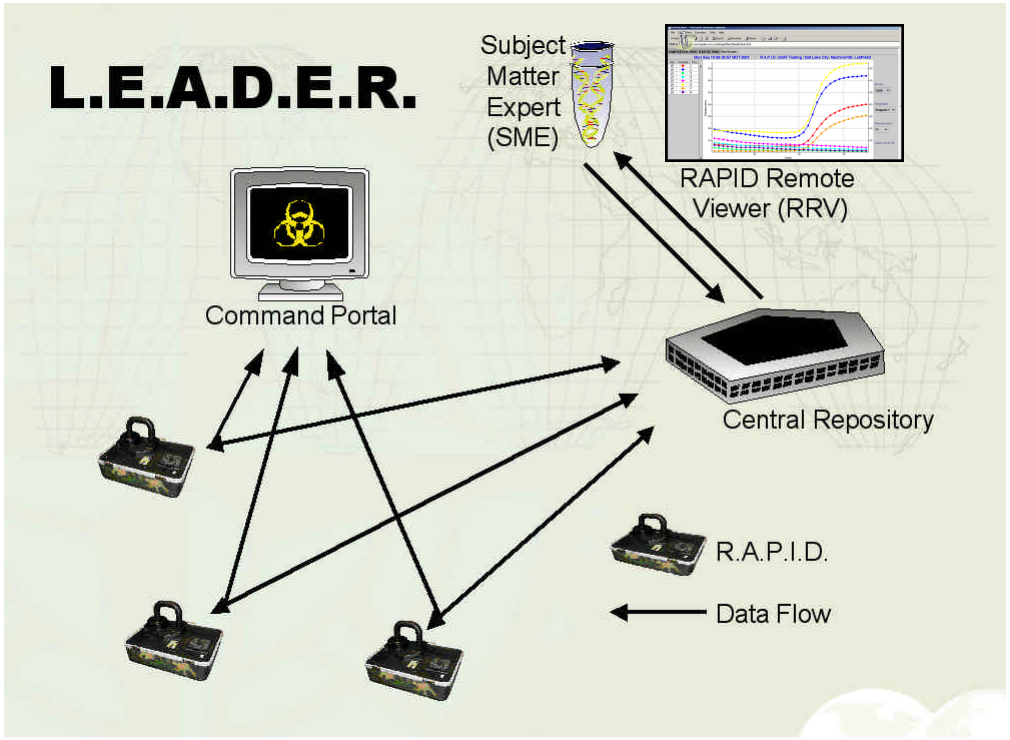
d. Test button configuration



Practice Run programming and link to Test button.

RRV / LEADER / Field Applications

NOTES:


L.E.A.D.E.R.
Subject Matter Expert (SME)
RAPID Remote Viewer (RRV)
Command Portal
Central Repository
R.A.P.I.D.
Data Flow

NOTES: _____

Notes: Open Practice (Sample Prep/Reagents/PCR/Pipetting / MMBWC CD-ROM)

NOTES:

Customer and Technical Service

Help process/Structure (How-to)

Contacts/Forms

Reach Us On the Web

http://www.idahotech.com/rapid

Reach Us By E-mail

support@idahotech.com - *Technical Support*
it@idahotech.com - *General Support*

Reach Us By Phone

For technical support call:
1-800-735-6544 - United States and Canada
1-801-736-6354 - Utah
IDD Prefix-1-801-736-6354 - International

Reach Us By Fax

1-801-588-0507 - *United States and Canada*
IDD Prefix-1-801-588-0507 - *International*

R.A.P.I.D. Users Web site

Netscape: Idaho Technology - R.A.P.I.D.
Idaho Technology
BioChem
R.A.P.I.D. Network
for authorized personnel only
LogIn
Username
Password
Customer Location
>> Login
Help! You forgot your username or password.
You need to obtain a username and password.
© Idaho Technology Inc. 2001 All rights reserved.
No part of this site may be reproduced without express written permission from Idaho Technology Inc.
THE THREAT IS REAL

NOTES:

General Maintenance

Cleaning:

Chamber and machine exterior: use 10% bleach solution (also for decontamination purposes).

Optics: use cleaning solution and lens paper provided in zip pockets.

Machine Service:

RMA forms and procedures

DECONTAMINATION FORM

1 Equipment Information

2 Customer Information

3 Decontamination Information

4 Authorization Notice

EQUIPMENT RETURN PROCEDURE

1 Returned Equipment

2 Decontamination Labels

REPAIR & RETURN FORM

1 Equipment Information

2 Customer Information

3 Decontamination Information

4 Authorization Notice

R.A.P.I.D. Tool Kit

NOTES:

Fuse Replacement

Before performing maintenance or troubleshooting on the R.A.P.I.D., turn off the machine, unplug the power and serial cables and allow the heating duct assembly to cool. Use the correct tools from the supplied tool kit and wear safety glasses and protective gloves.

The fuse assembly is located next to the power switch.

1. Place a flathead screwdriver in the small slot to pop the fuse assembly out of the mounting hole. The fuse assembly will pop up when the plastic latch is released.

2. Replace with properly rated fuses. Insert assembly into mounting and snap it in place.

The R.A.P.I.D. type is identified by the serial label that is on the main panel.

NOTES:

Notes: Open Practice Time

NOTES:

Final Unknown Run / Review

NOTES:

R.A.P.I.D. Accessories

Vendor	Item	Catalog Number	Price for each item
Idaho Technology			
	IT BioReagents	TBD	$6.50 per assay
	Manuals	TBD	$12 per O&T, CDs
	IT 1-2-3 DNA Kits	TBD	TBD
1-800-735-6544 / www.idahotech.com			
Roche Molecular			
	Capillary tubes	1 909 339	$57.62 per 96
	PCR template prep kit	1 796 828	$151 per kit (100)
1-800-428-5433 / www.lightcycler.com			

Vendor	Item	Catalog Number	Price for each item
Rainin			
	P-20 pipettor	P-20 (2-20uL)	$244.50
	P-200 pipettor	P-200 (50-200uL)	$244.50
	small tips with barrier	RT-20F	$85 per 10 racks
	large tips with barrier	RT-200F	$85 per 10 racks
1-800-472-4646 / www.rainin.com			
ISC			
	Gloves	Size	$10.35 per box
1-800-999-2901 / www.bioexpress.com			
Bio-Spec			
	Mini Bead-beater screw-cap tubes/beads		
1-800-617-3363 / www.biospec.com			

NOTES: